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An outbreak report on identification and pathological changes associated on natural case of NDV

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Abstract

The outbreak report concise the key aspect of recent identification and pathological changes associated on natural dead due to Newcastle disease (ND). The outbreak, caused by the avian paramyxovirus, has posed significant challenges to poultry populations and huge economic loss worldwide. This study conducted at the Department of Veterinary Pathology, College of Veterinary Science, A.A.U., Khanapara, Guwahati-22 from an outbreak in broiler flock located in the kamrup district of Assam with a capacity of 400 number of birds which were well maintained under intensive system. Upon internal necropsy examination respiratory organs consistently revealed haemorrhagic tracheitis and congestive pneumonitis in the upper and lower respiratory tracts respectively. Major consistent gross findings were petechial haemorrhages in the proventriculus and caecal tonsils. Histopathological picture of all the visceral organs consistently revealed presence of extensive vascular changes characterized by congestion and haemorrhages and cellular changes characterized by inflammatory cellular infiltration. Following the clinicopathological studies the representative samples from all the suspected cases were subjected to the HA, HI, and molecular detection of complete F gene of NDV by RT-PCR.

Keywords: RT-PCR, ND, NDV, ICTV, H&E

Introduction

Poultry is one of the major segments that contributed significantly to the livestock revolution and food production. Poultry encompasses domesticated avian species such as chicken, ducks, quails, turkeys and guinea fowls which are being raised for eggs, meat and feathers. Indian livestock sector contributes 4.19% in National GVA and 28.63% in the agricultural GVA as per the National Accounts Statistics in 2018-2019 (Anon, 2021). With 180 million eggs produced annually, India ranks third in the world for both egg production and chicken meat production, accounting for 3.10% of the world's poultry production. As per the 20th livestock census the total Poultry population in the country is 851.81 million in 2019 among which 30% were layers and 40% were broilers. Assam state is surrounded by Manipur, Mizoram and Tripura as neighbouring states and shares international borders with Bangladesh and Myanmar. This often increases the incidence of epidemics of emerging and re-emerging avian diseases such as Newcastle disease that severely affect the sustainability of production. As the people in Northeastern-region depend on chickens for their food and because it is geographically close to China, Myanmar, Bangladesh, and Bhutan, where newer strains of NDV have been reported, ND has emerged as a serious concern in North Eastern India. (Wu *et al.*, 2015; Nooruzzaman., *et al.*, 2013; Henning., *et al.*, 2013; Henning., *et al.*, 2009; Zhang., *et al.*, 2012) [15, 14, 8, 7, 16]. Newcastle disease was formerly known as Ranikhet disease, after the region of India where it first appeared. Both wild and domestic birds all over the world are affected greatly by Newcastle disease. (Amarasinghe *et al.*, 2018; ICTV, 2019; Miller *et al.*, 2013) [1, 9, 13]. Newcastle disease stood at 3rd position amongst the most important diseases in the domestic birds having been reported in 109 member countries of World Health Organisation of Animal Health (OIE). (Anonymous, 2022) [3].

Outbreaks of ND in commercial poultry and backyard birds have been reported from many parts of India despite of stringent vaccination practices in. (Mariappan *et al.*, 2018)^[11]. As there are so many commercial vaccines on the market, the disease can have a significant impact on the destruction of poultry farms around the world and can be controlled by using the vaccinations strictly according to the schedule. (Mebatsion. *et al.*, 2002)^[12]. The widespread distribution of the virus may be due to the persistent emergence and re-emergence of the virus with notable genome mutations. Developing nations may be affected by strains of the virus with high virulence, and traditional live vaccines appear to be less effective. This might be the result of improper cold chain and storage conditions. The issue is made worse for the production of poultry in tropical nations. The main causes of its outbreak in developing countries are the emergence of newer strains and the ineffectiveness of vaccination. (Kumar, 2015)^[10]. The proper recognition and identification of the disease is crucial because these high consequence variants have the potential to have a significant economic impact.

Materials and Methods

The present study on was conducted at the Department of Veterinary Pathology, College of Veterinary Science, A.A.U., Khanapara, Guwahati-22 from an outbreak in broiler flock located in the kamrup district of Assam with a capacity of 400 number of birds which were well maintained under intensive system. In the study samples were collected from dead carcass of broiler flock presented for post mortem at the Department of Pathology of College of Veterinary Science, Assam Agricultural University, Khanapara. The birds presented to post mortem were examined grossly and Representative tissue samples were aseptically collected from the proventriculus, caecal tonsils, spleen, liver, bursa of Fabricius, lungs, and trachea in sterile container and fixed in 10% buffered formalin for histopathological examination as per procedure of Luna, 1968. Suspected cases were further screening by serological tests such as Haemagglutination (HA) assay and

Haemagglutination inhibition (HI) and confirmatory diagnosis was done by molecular test using RT-PCR in serologically positive samples as per the recommendations by Deka *et al.*, 2022^[5].

Results

A detailed necropsy examination was conducted on 26 number of broiler birds out of 400 number capacity broilers presented for post mortem at the Department of Pathology of College of Veterinary Science, Assam Agricultural University, Khanapara. On post mortem examination showed haemorrhagic tracheitis and the mucosa of the trachea was covered with catarrhal exudates mixed with blood. The proventriculus of the affected birds revealed the presence of marked petechial and ecchymotic haemorrhages at the tips of the proventricular gland and caecal tonsils were found to be haemorrhagic (Fig. 1A and 1B). Spleen in all the affected birds were found to be friable and mottled in appearance (Fig. 1C). Microscopically, trachea showed mild congestion and tracheitis with mononuclear infiltrating cells along with sloughing of mucosa and mucosal epithelial cells (Fig. 2A) in majority of cases and proventriculus shows haemorrhages in the lumen of glands (Fig. 2B). Loss of demarcation between red and white pulp, foci of hemosiderin deposition and loss of germinal centres were observed in spleen and also haemorrhages in the caecal tonsils (Fig. 2C). All the 26 birds that were suspected for ND by gross and histopathological examination were further tested using HA and HI serological assays. The samples that gave positive haemagglutination reaction in HA assay (Fig. 3A) were further tested by HI assay (Fig. 3B) and the titre was recorded at the highest dilution where there was haemagglutination inhibition. The positive samples in HI assay were then processed for molecular detection by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) using the oligonucleotide primers targeting F gene of NDV and were found positive for Newcastle disease virus with amplification product of 363 bp.

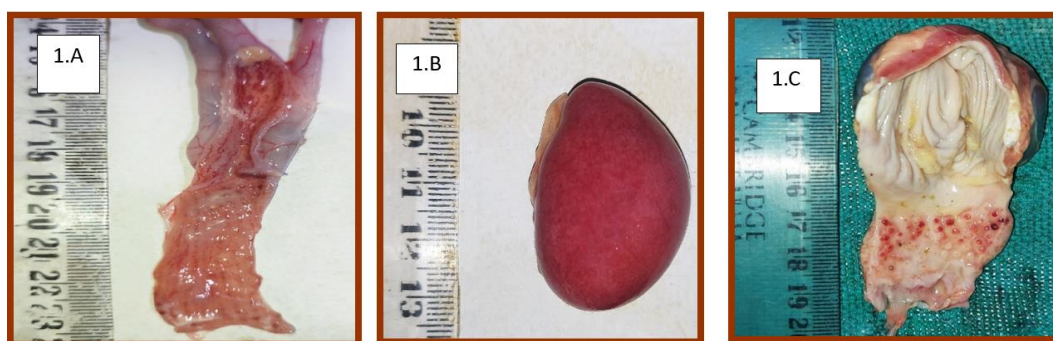


Fig 1: A: Showing Haemorrhages in the Proventricular Glands. 1. B: Showing Necrosis and Haemorrhages in the Small Intestine and Caecal Tonsil. 1. C: Showing Splenomegaly and Mottled Spleen

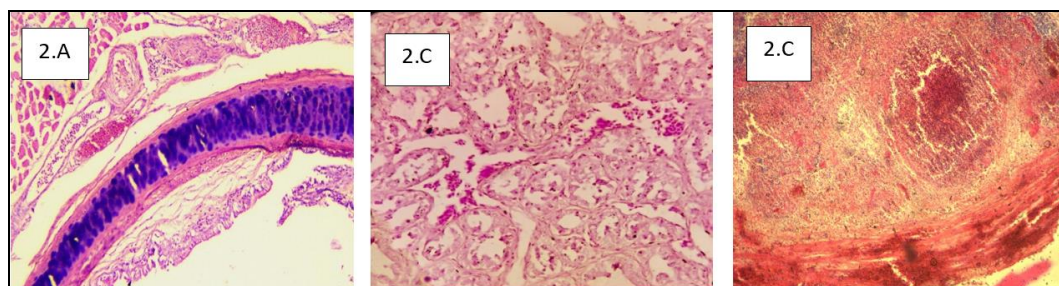


Fig 2: A: Photomicrograph Showing Mild Tracheitis with Infiltration of Inflammatory Cells, Loss of Ciliated Epithelium and Congestion, H&E X10. 2. B: Photomicrograph of Haemorrhages in the Proventricular Glands, H&E X40. 2. C: Photomicrograph Showing Haemorrhages in the Caecal Tonsil H&E X40.

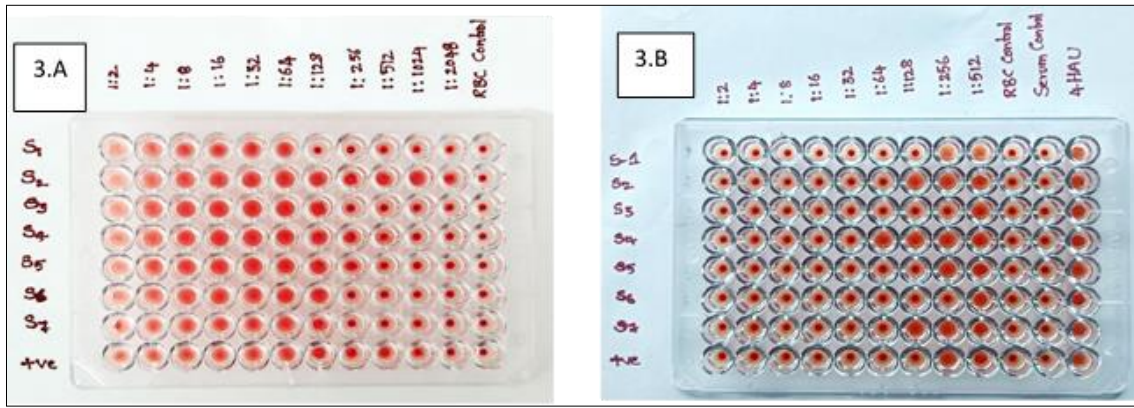


Fig 3: A: Photograph of Ha Test. **3. B:** Photograph of Hi Test

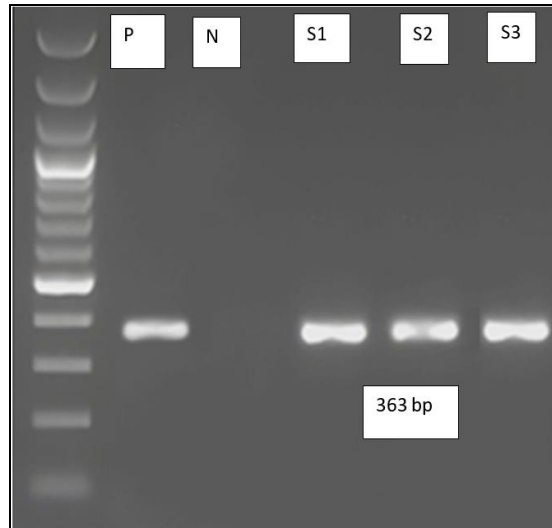


Fig 4: Showing P for Positive Control, N for Negative Control, S1 to S3 Field Samples Shows Positive Isolates by Pcr With Band Size of 363bp on Agar Gel Electrophoresis.

Discussion

Grossly, Haemorrhages were present on glandular surface of proventriculus, in the mucosal surface of intestines and caecal pouches, on the surface of spleen and liver and heart. In some cases, liver was congested and enlarged, lungs were congested and occasionally pneumonic and trachea showed congestion. In few cases there were congestion on the brain. All the gross changes were in agreement with the findings of the previous workers (Etriwati *et al.*, 2017; Mariappan *et al.*, 2018; Ansori *et al.*, 2021) [6, 11, 4]. Histopathological findings include haemorrhages in proventricular glands and intestines, sloughing of intestinal villi, necrotic areas in the caecal tonsil, sinusoidal congestion were coincided by earlier workers (Etriwati *et al.*, 2017; Mariappan *et al.*, 2018; Ansori *et al.*, 2021) [6, 11, 4] with varied degree of intensity. All the samples that were found positive in HA and HI assays were tested by RT-PCR for confirmatory diagnosis as per the recommendation of the OIE terrestrial manual (2018). The oligonucleotide primers targeting F gene (Deka *et al.*, 2022) [5] were used in RTPCR to obtain amplified product of Fusion protein of ND virus with 363bp (Deka *et al.*, 2022) [5]. All the samples were found positive through RT-PCR and these findings were similar to the findings of Deka *et al.* (2022) [5] with varied positivity rate in a study on backyard and commercial poultry in Assam.

Conclusion

The most consistent gross findings in upper and lower respiratory tracts of NDV affected flocks were exudation in

trachea and haemorrhagic tracheitis extensive consolidation. Slight splenomegaly was found and the organ had whitish patches on the surface and turned dark red in colour in some birds and Caecal tonsils of almost all the birds revealed petechial haemorrhages and necrosis in the area. Microscopically, caecal tonsils presented haemorrhages and inflammatory cell infiltration in the mucosal and the interstitial region. There was presence of extensive diffuse and multifocal areas in the proventriculus and spleen presented deposition of hemosiderin at a few areas and loss of germinal centres in the parenchyma. There was a loss of demarcation between the red pulp and the white pulp.

Conflict of interest: None

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